suspended in the streams passing over and through marshes, swamps, bogs and deltas, and are so divested of any destructive power upon bone. And in any case, the elaboration of these acid products which we are considering would be partial or completely suspended at such depths as are usually given for the repositories of vertebrate remains. Yet, however diverted or minimized may be the action of organic acids and carbonated water upon bone, there can be little doubt that it is considerable, and an important means in many cases of imparting to them much fragility or of entirely disintegrating them.

(*To be Continued.*)

THE BACTERIAL DISEASES OF PLANTS: A CRITICAL REVIEW OF THE PRESENT STATE OF OUR KNOWLEDGE.

By Erwin F. Smith.

(*Continued from p. 804*)

IV.

II. THE HYACINTH (HYACINTHUS ORIENTALIS).

(II) The organism: *Bacillus hyacinthi* (Wakk.) Trev. (1883).

1. *Pathogenesis:*

(A) Yes.

(B) Yes (?). The poured plate method was not then in general use. Inoculations were made directly from diseased plants into sterile nutrient fluids, or into tubes of nutrient gelatin, and the resulting cultures may not always have been pure ones, although the writer's own experience has shown conclusively, in case of melon wilt—a somewhat similar disease—that it is often possible to obtain pure cultures in this way, if the culture

media is sterile to begin with and the necessary precautions are taken to exclude surface contaminations and air-borne germs. His experiments were, however, checked and controlled by means of poured plates, whereas Dr. Wakker had advantage of no such exact method. Nevertheless, he seems to have worked with great care, and states positively that although the bacteria were often transferred from diseased plants to the culture media, and also from one tube of media to another, the results were always the same, which could scarcely have been the case were intruding organisms present.

(C) Yes (?). Infections with artificial cultures had not been secured up to March, 1895, and do not appear to have ever been very numerous or very successful. The only experiment which seems to come properly under this head was begun March 4, 1886. The inoculations were made from a liquefied gelatin culture, the fluid being inserted into fresh cuts on the scapes of several (more than five) varieties of hyacinths. In a week all of the scapes began to dry out and soften, from the summit downward; and fifteen days later the greater part of each one was either entirely dry, or soft and flacid. An earlier effort to infect from a bouillon culture failed (Verslag, 1884).

(D) Yes; in part. On microscopic examination of the scapes mentioned under *C* it was easy to determine in them the existence of the yellow disease; but this did not extend into the bulbs. "These experiments [referring to those mentioned under I (5) as well as this one] were repeated and varied with, in general, concordant results."

Conclusion.—Pathogenic nature rendered probable.

Remarks.—As will be seen later on, this organism was imperfectly described, and any bacteriologist having opportunity to repeat and extend Dr. Wakker's experiments should by all means embrace it.

2. *Morphology:*

(1) *Shape, size, etc.*—The bacteria which Dr. Wakker regards as the cause of this disease are represented on his Plate I, Figs. 1–8 (**34**). They are two to four times as long as broad, with an ordinary length of about 2.5 μ. Their form is therefore more or less that of a cylinder, but with rounded ends. They are said to agree tolerably well in size and shape with *Bacterium Termo*. The organism was described as *Bacterium Hyacinthi* in 1883, but was placed under *Bacillus* by Trevisan in 1889. When these bacteria have been in a nutrient liquid for some time a certain number become longer than they were, and now measure 4 μ, while the ordinary length is only 2.5 μ. Later on, as the nutrient matters of the liquid are becoming exhausted, the bacteria diminish in size more and more, and gather into motionless groups, which often have circular outlines and which grow by the accession of new individuals, while the motile bacteria become less and less numerous. Bacteria from the dry slime were found to be only about half the ordinary size, but on placing them in nutrient fluids they resumed their normal size. Examinations were made in hanging drops of nutrient fluid.

(2) *Capsule.*—No mention of any capsule.

(3) *Flagella.*—No mention of flagella. The organism is said to be actively motile in culture fluids. Even those kept for some time in a dry state are said to have acquired motility on placing them in nutrient fluids. In the yellow, viscid slime, as taken from the plant, they are not motile; but motility begins as soon as this is diluted with a $\frac{3}{4}$ per cent. salt solution, or with a suitable nutrient fluid. "After a short time all is life and motion; the bacteria, in the form of straight but very flexible rods, are to be seen moving about actively; individuals in repose are rare. Among the undivided bacteria there are many which are in process of division, and which then show two individuals moving together; these, however, soon separate to continue an independent existence." It will probably be found that the organism is also motile in the plant in early stages of the disease, *i. e.*, before it has multiplied to such an extent as to fill the vessels. Dr. Wakker himself says: "It is evident that

though they exhibit no visible motion in the slime they cannot be entirely without motion in penetrating into the bulb."

(4) *Spores.*—The bacillus produces endospores. Their development and germination was followed with so much care that it appears worth while to give a somewhat detailed account.

"In the cultures already described [those at room temperatures] no spores were found. These had, therefore, to be sought in some other way. They were finally obtained from the liquid cultures by keeping them at a higher temperature. Drops of nutrient fluid containing the bacteria were placed in an enclosure having a uniform temperature, night and day, of 35° C., *i. e.*, at a temperature exceeding the mean temperature of the room by about 20° C. Ordinarily, at the end of ten days, spores appeared, and the characteristic agglomerations of small, motionless individuals did not appear. Subsequently it was found that a temperature of 35° C. was not absolutely necessary for the formation of the spores. In fact, during the summer, when a rather high mean temperature prevailed in the room, some cultures produced spores without artificial heat; but these were never as numerous as those formed in the tubes kept at the uniformly higher temperature of the enclosure. The spores of *Bacterium Hyacinthi* (**34**, pl. I, fig. 1) have that lively bluish brilliancy which is usually so characteristic of the spores of bacteria, and which is caused by the strong refraction of the light. These spores are always a little longer than broad, and form in the interior of the largest rods near the middle, although ordinarily slightly nearer one of the extremities. In consequence of their strong refrangibility it is difficult to decide with certainty whether the rod is swollen around them, as has been indicated for several similar species. This swelling, if it exists, must be very slight, since the spore is not thicker than the bacterium itself. Besides the rods with ripe spores, there are, ordinarily, a great number engaged in forming spores, and these still move about in a lively manner. On the contrary, when the spores have reached full development, the rods which contain them remain motionless and their wall soon disappears, so that the spores become completely free. In this state they

are about 1 μ in length, while their breadth does not exceed $\frac{1}{2}$ or $\frac{2}{3}$ of this size. Each rod produces only one spore. If these spores are allowed to dry on the glass where they have been formed, they may be kept for a long time, and subsequently on placing them in a nutrient liquid the development of new bacteria may be observed. At the time of germination, which is hastened the same as sporogenesis, by an increase of temperature, the spore begins to swell and its cylindric form changes to an ellipsoid. The strongly refractive power is also gradually lost, the middle of the spore first becoming dull while the brilliant gleam still persists more or less at the two extremities (34, pl. I, figs. 2 and 3). Here we have the condition which must be considered as the commencement of germination. The wall is split into two portions, which remain united at one side. The central part of the spore from which the refringence has entirely disappeared, is the place where the two halves of the spore open one from the other, and here a baculiform body of slight refringence was observed pushing out (pl. I, figs. 4 and 5). During the growth of this body the sheen also diminishes very greatly at the two extremities of the spore, and soon there is a state which cannot be indicated better than by likening the germinating spore to a hammer, the two portions of the wall of the spore representing the head while the handle is formed by the rod which has issued from the spore (fig. 6). Often after a longer or shorter time, the rod, one end of which is squeezed between the two parts of the wall of the spore, begins an oscillatory movement, and thus succeeds in freeing itself, whereupon it moves through the liquid in the manner common to bacteria, the empty wall being left behind (fig. 6c). In other cases, after escaping from the spore, the young bacterium remain motionless in front of the empty wall for a long time before swimming away. Finally, the rod sometimes drags the empty wall after it (fig. 8). In all cases the rod which has escaped is an ordinary bacterium which soon divides in the manner already described."

The author never found spores in the living hyacinth. This, he says, accords with de Bary's observation on *Bacillus anthracis*, he having never found spores in the living animal.

This absence of spores in the living plant is also in harmony with the fact that in the nutrient liquid the formation of spores begins only when the alimentary substances are exhausted. This, naturally, is never the case in the living bulb. It is not impossible, however, that when diseased bulbs have been entirely destroyed spores may form in the remaining mass if the temperature is favorable. An effort was made to prove that these spores were actually developed from the hyacinth bacillus by allowing a drop of fluid containing them to dry on a slide for some time, and then placing that part of the slide bearing the dry spores in contact with the fresh cut surface of a bulb. In three weeks the yellow disease was discovered in the vessels of the bulb, and it was at once apparent that it had already been developing in these for some time. This experiment was repeated several times, and always with the same result. This, indeed, is not full proof; but when old cultures are used very few vegetative rods are left, and the infection is believed to have resulted principally from the germination of the spores in the sticky fluid that oozes from the cut scales, the bacteria finding their way from this into the vessels.

(5) *Zooglœa.*—No special mention of zooglœa. Possibly the more or less circular or globular groups of motionless rods which commonly appeared in the cultures as they became exhausted are to be regarded as such.

(6) *Involution forms.*—No mention of any involution forms.

3. *Biology.*

(1) *Stains.*—This organisms stains very readily in the most diverse analin colors. The author made a variety of experiments to determine the best method of staining the bacteria in place in the tissues. He obtained the best results with analin browns, especially phenyline brown (Bismark brown), but states that many other colors may be used, *e. g.*, eosine, methyl violet, analin yellows and picric acid. The yellow stains have the special advantage of giving to the preparation almost exactly its natural color. To stain in place, sections made from alcoholic material should be put into a saturated alcoholic solution of the analin brown, left for a few minutes, and then transferred to strong alcohol containing an

extremely small quantity of hydrochloric acid. In this the color rapidly disappears, especially if the fluid is stirred with a glass rod. At the end of a very short time, the length of which varies in different cases, certain parts will be seen to have preserved their color, if the disease is present, while the rest of the section has already bleached. The sections must now be removed immediately to a dish of pure, anhydrous spirits of turpentine, in which they are left until thoroughly penetrated by the liquid. They may then be examined directly or first mounted in Canada balsam, after which they may be kept indefinitely. When the work has been well done the sections will be brown in those parts which contain the bacteria and which were originally yellow, while in all other parts they are colorless.

(2) *Gelatin.*—The culture media was made by adding to water containing glucose and a little meat extract, enough gelatin to give a solid, clear yellow, perfectly transparent mass at ordinary temperatures. This was sterilized by heating from time to time to 100° C. It was then carefully pipetted into tubes which were plugged with cotton, and re-sterilized by heating every day to 100° C., for some days. Pipettes, tubes and cotton plugs had previously been heated to 140° C. Tubes prepared in this way were unplugged, infected with bacteria taken from a diseased bulb (the transfer being made by means of a platinum wire previously heated to redness), quickly closed, and then left at the ordinary room temperature. The organism makes a good growth on gelatin. The gelatin is readily and completely liquefied.

"*Experiment of June 12, 1885.*—The above described operations were made this day, and two days later I saw in all the tubes the gelatin liquefy under the influence of the bacteria. Examination showed that the part not yet liquefied also contained bacteria, so that the latter must first penetrate into the gelatin and then cause its liquefaction. The formation, in the part of the gelatin which is still solid, of white globules consisting entirely of bacteria, served to make this fact very apparent. Bubbles of gas which can only arise from the action of these organisms also developed in it continually. After a short

time the whole mass was liquefied, and the bacteria were found at the bottom of the tube as a thin whitish layer. The liquid is then a clear brown, darker than the original gelatin. The contents is almost odorless.

"This experiment was repeated very often, and always gave the same result, only in subsequent experiments, it happened sometimes that the white globules did not appear. This, however, is not surprising, since I then employed a mixture (glucose, extract of meat, gelatin) of slightly different composition, and since, moreover, the temperature was not always the same. On peut naturellement infecter aussi quelques tubes au moyen de Bactéries prises dans d'autres tubes; cela n'a jamais rien changé aux résultats."

No gelatin roll or plate cultures were made, and the behavior of the organism in stab and streak cultures is not carefully described.

(3) *Agar.*—No account of any experiments on agar media.

(4) *Potato, etc.*—Nothing mentioned.

(5) *Animal Fluids.*—The first artificial medium was made by adding a little meat extract and grape sugar to a decoction of meat which had been kept for some time in spirits, and was freed from the latter by washing and boiling in distilled water. It was then boiled for an hour in an additional quantity of distilled water and the sugar and meat extract added. It remained clear for ten days, was then reboiled, cooled quickly, and a small quantity of the yellow slime introduced, the greatest care being used throughout to avoid contaminations. The second day this fluid became distinctly clouded, and this clouding increased for four days, and then remained the same. The inoculations that failed were from this culture. The slime used to infect this culture came from a single vascular bundle of a freshly cut bulb. It was scraped off on a flamed cover-glass, which was then thrown into the fluid. The organism also grew well in a solution of meat extract to which glucose had been added. This was the fluid culture medium ordinarily employed, and there is no mention of any other. The exact composition of the medium is not given.

(6) *Vegetable Juices.*—None mentioned.

(7) *Salt Solutions and other Synthetic Media.*—No mention of any; but since the organism is not strictly parasitic it is inferred that it can grow and maintain itself for a long time in a variety of organic substances.

(8) *Relation to Free Oxygen.*—The organism is aerobic and probably also facultative anaerobic, although no mention is made of any experiments to determine this point.

(9) *Reducing and Oxidizing Power.*—Peptonizes gelatin.

(10) *Fermentation Products and other Results of Growth:*

(a) *Gas Production.*—Organism produces gas in meat extract gelatin containing grape sugar. Kind of gas not determined.

(b) *Formation of Acids.*—No statement.

(c) *Production of Alkali.*—No statement.

(d) *Formation of Pigment.*—In the vessels of the plant the organism produces a bright yellow color, which is soluble in glycerin, but insoluble in water and alcohol. This pigment became darker on drying. The dextrose, meat extract gelatin became darker colored (clear brown) after liquefaction.

(e) *Development of Odors.*—The organism produces little or no odor either in the plant or in the artificial cultures. This absence of odor may be used to distinguish the disease from other hyacinth diseases, some of which are very malodorous.

(f) *Enzymes.*—Evidently not studied. Organism produces at least two; one capable of peptonizing gelatin, and another which dissolves the cellulose of the hyacinth.

(g) *Other Products.*—None mentioned.

(11) *Effect of Dessication.*—The organism can be kept for a long time in a dry state without dying, *e. g.*, on a glass plate. It shrinks to about one-half normal size, but on placing again in suitable fluids it recovers its former size and makes a new growth. One of these hanging drop cultures was begun in a somewhat different way. The bacterial slime was not taken directly from a bulb but from a glass plate on which it had been placed and dried long before. The slime and the nutrient fluid were then mixed in the same manner as before; but instead of rods 2.5 μ long, the bacteria were now smaller. Moreover, at first they were distributed through the liquid passively,

and a longer time passed than in the other cultures before their own movement appeared. Nevertheless, after some hours, it began, and first as a simple rotation. At the same time it was determined that the dried bacteria had been reduced to about one-half the ordinary size. But the following day they had resumed the ordinary size, and then also showed the characteristic backward and forward movement. From this point on the culture presented the identical phenomena described above. This shows that the bacteria of the *maladie du jaune* can live for a long time in a dry state, and that on drying they are reduced to dimensions comparable to those which they assume in a liquid in which the alimentary substances are becoming exhausted. I infer that this dry mucilage did not contain spores.

(12) *Thermal Relations:*

(a) *Maximum for Growth.*—Not determined.

(b) *Optimum for Growth.*—Not determined. The organism grows at living-room temperatures, and also in the thermostat at 35° C.

(c) *Minimum for Growth.*—Not determined. The natural progress of the disease in the hyacinth fields appears to be slow, and probably low temperatures may have something to do with this.

(d) *Death Point.*—Not determined.

(13) *Relation to Light.*—Not determined.

(14) *Vitality on Various Media.*—Seems to be capable of living for a considerable period in various media.

(15) *Effect on Growth of Reaction of Medium (acid, neutral, alkaline.*—No statement.

(16) *Sensitiveness to Antiseptics and Germicides.*—No statement.

(17) *Other Host Plants.*—No mention of any. Some speculation as to origin of the disease, but no facts.

(18) *Effect upon Animals.*—No statement. Probably not tried.

(III) ECONOMIC ASPECTS:

(1) *Losses.*—No statement as to the extent of damage done by this disease. The disease is spoken of in one place as the chief subject of his investigations, and in another place the organism is called a " dangerous parasite."

(2) *Natural Methods of Infection.*—Little that is definite can be gathered from Dr. Wakker's writings. The sticky slime which oozes from rifts in the affected leaves is highly infectious, adheres to whatever it touches, retains its vitality for some time, and is readily borne about on light objects. He discusses the possibility of the germs entering through the blossoms, and considers that wounds are more likely sources of infection, because the attacked blossoms would fall off quickly and carry the germs with them. It probably enters the plant through wounds made by man or animals. Dr. Wakker thinks it especially likely to enter through wounds of the scape made in cutting the flower, or through injuries done to the young scales by pulling leaves, or by cutting healthy bulbs with infected knives in process of making incisions in the bulb, or of separating the scales for purposes of reproduction. It is evident, however, from the fact that the greater number of the plants are first attacked at the tip of the leaf, that some other unknown method of infection is the more common one. Dr. W. thinks the infection often takes place very early in the spring and generally through the air, the sticky bacterial exudate from the leaves, etc., being carried to sound plants by wind and rain, or by flies and other insects which frequent the hyacinth fields on warm days (Verslag, 1883). For various reasons Dr. Wakker thinks that the parasite may sometimes enter through the uninjured leaf, *i. e.*, through the stomata, but does not appear to have induced the disease in this way. Wounds are always moist, and the bacterium finds food ready for its use in the dead cells of the wound, whereas if it enters through the stomata it must make its own food from the start. The stomata are also very small, and infection through the uninjured leaf surface is probably uncommon.

(3) *Conditions Favoring the Spread of the Disease.*—Dr. Wakker states that the spread of the disease is favored by wet weather, and that dry weather and continuous sunshine are the best preventives. If the much lessened prevalence of the disease in 1883 as compared with 1882 is to be attributed in part to the precautionary measures taken, it is not less certain that the frequent rains of 1882 did great injury to the plants in this

particular. "In 1883 innumerable were the cases in which I observed that the descending stripe on the leaves was dried out and stopped, so that the bulb was not attacked." The rapidity of the infection depends largely on the temperature, the dampness in the surrounding air, and on the amount of water in the plant itself. The location of the wound might also make a difference.

(4) *Methods of Prevention.*—An inquiry among the growers elicited the statement that there is a great difference in susceptibility. This Dr. W. thinks cannot be denied. Some varieties are very subject; others, in the same beds or gardens, have not been known to be attacked. Many varieties formerly held to be exempt from the disease are now known to be subject; but some remain which have never yet shown the yellow disease, and this cannot be ascribed to mere accident; on the contrary, it can be explained only by assuming that predisposition or readiness to be attacked here plays a prominent part (Verslag, 1883). Anatomically, so far as known, all are alike. Lists of "very susceptible," "less susceptible" and "not susceptible" varieties are given, from which it would appear that single varieties are more susceptible than double ones, and the exemption of the latter is not due to their lesser number. All of the double red varieties and most of the other double sorts are exempt, or but little subject to attack. These lists are based on statements furnished by only seven growers, but include many varieties (Verslag, 1885). Of thirteen varieties said to be very susceptible by several or most of these seven growers only one is double, la Tour d'Auvergne. On this account, difference in receptivity is suggested as a means of combatting the disease. New varieties must not be originated from susceptible ones. Seedlings should be derived from hardy sorts, and by artificial fecundation, the pollen of susceptible varieties being excluded. Otherwise, through the agency of insects, the resulting cross may prove susceptible. The law of heredity is shown still more rigorously in non-sexual reproduction. It is best, therefore, to discard sensitive sorts and try to obtain new ones which are more robust.

In the division of bulbs for propagation the greatest care should be taken never to cut a healthy bulb with a knife which

has been in contact with a diseased plant, at least not until it has been disinfected.

There is another point to which the author desires to call special attention, viz., to the removal of leaves which begin to show signs of the disease at the tip. On May 20, 1883, the diseased leaves were entirely cut away from seventeen hyacinth plants. On September 26th, sixteen of these bulbs were entirely sound, although rather small. The other bulb was entirely decayed; but from what cause, it was no longer possible to determine. Planted in pots these sixteen bulbs blossomed in April, 1884. The following June they were dug up once more, and on cutting them open all were found to be sound. This experiment was tried on many other bulbs, and always with the same success. It was also tried by several horticulturists in their fields with results entirely confirmatory. It is, therefore, certain that the bulb can be preserved by the judicious removal of diseased leaves.

Since the bacteria have always penetrated much further into the leaf than is to be seen with the naked eye, the whole leaf should be removed even when only slightly attacked. The frequent complaint that cutting off the diseased parts does no good, shows that not enough attention has been paid to this. Of course, when the bulb is already infected, cutting off the leaves amounts to nothing (**31**).

Finally, it goes without saying that the debris of diseased hyacinths should not be left in the field or near it, as one might be tempted to do on account of its value for manurial purposes. All such debris should be thrown into a deep ditch and disinfected with quick lime.

Remark.—Considering the time when this piece of work was done, it is remarkably good, and in all of the papers cited the internal evidence indicates a careful, conscientious, brilliant investigator. There can be no doubt that the disease is due to a bacterial parasite; but to complete the proof that the disease is due to the specified organism it should be obtained by infections with pure cultures obtained from single colonies. The organism thus isolated should also be studied under a wider range of artificial conditions than were employed. Indeed, excluding the pathogenic test, it is more than doubtful if the organism could be identified from the description.